THE OXYGEN CONSUMPTION OF ARTIFICIALLY ACTIVATED AND FERTILIZED CHÆTOPTERUS EGGS

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Introduction

It is well known that unfertilized *Chætopterus* eggs, when they are treated with various physical or chemical agents, undergo a special type of development (F. R. Lillie's (1902, 1906) differentiation without cleavage). Lillie's original observations have been recently confirmed and completed by Pasteels (1934) and by myself (1937). These investigations show that if the unfertilized eggs are treated with 5 per cent isotonic KCl in sea water, they first complete their maturation; immediately after the expulsion of the second polar body, they start undergoing a series of monaster cycles. During each of the cycles, there is an increase in the number of the chromosomes and the appearance of lobulations simulating cleavage. But the furrows fade out quickly and the egg resumes its original shape. There is, therefore, a considerable increase in the size of the egg nucleus and in the number of the chromosomes during the first hours of differentiation without cleavage. Later on, the big single nucleus breaks down and, at the same time, occur processes comparable to gastrulation; in the best cases, unicellular ciliated eggs, resembling somewhat trochophores in their general shape, can be obtained.

It seemed of interest to measure the oxygen consumption of eggs undergoing differentiation without cleavage and to compare it with the respiration of unfertilized eggs: it was hoped that such an investigation would throw some light on the question whether energy is needed for cleavage and differentiation, a problem which has recently been investigated by Tyler (1933, 1936) on other eggs.

A beginning was made last year during a stay in the Zoölogical Laboratory in Naples and a report has been published recently (1937). The results, however, were far from satisfactory because a small percentage only of the eggs could be fertilized, although differentiation without cleavage was easily obtained. Lacking the necessary controls, we could only compare the data obtained for the activated eggs with the results published by Whitaker on the respiration of fertilized eggs in Woods Hole. Obviously, no definite conclusion can be drawn from

such a comparison and it was therefore necessary to reinvestigate the problem.

Material and Methods

The experiments were carried out following the technical details pointed out by Whitaker (1933): one or two ripe females were allowed to shed after the tip of their parapodia had been cut with scissors. The eggs were then washed repeatedly (5 or 6 times) in sea water in order to remove the mucus and a suspension of a concentration varying from 1 : 25 to 1 : 40 was prepared by slightly packing the eggs in a graduated centrifuge-tube and adding the required amount of sea water. Aliquot parts of the suspension (2 cc.) were pipetted into Warburg manometer conical cups (ca. 10 cc. capacity). Fertilization with one drop of diluted sperm or activation with 0.1 cc. of isotonic KCl usually occurred just before attaching the vessels to the manometers. Readings were taken after a 15-minutes equilibration period. The water-bath was kept at 24.8° and the manometers were shaken at a speed of 50 round-trip oscillations per minute with an excursion of 4 cm. That these conditions may be considered as adequate is indicated by the fact that increase in the speed of shaking induced no increase in the oxygen consumption; furthermore, the respiration of unfertilized eggs usually kept constant during 6 or 7 hours and they showed at that time little, if any, cytolysis.

Results

We know since Whitaker's experiments that there is a strong drop in the oxygen consumption of *Chætopterus* eggs at the time of fertilization. My previous experiments from Naples indicated that a similar drop occurs in KCl-activated eggs. In most of those experiments, activation was produced by tipping the KCl contained in the side-arm into the main part of the vessel after the respiration had already been measured during 1 or 2 hours. The results at Naples could be easily confirmed at Woods Hole: while the metabolism of the fertilized eggs dropped to 53 per cent of the initial level (average from 14 experiments in which the percentage of fertilized eggs exceeded 90 per cent), the oxygen uptake of the activated eggs (from the same females) fell to 51 per cent. There is therefore no doubt that, like the increase in respiration which follows fertilization in sea-urchin eggs, the drop observed in the present case is linked to the cytoplasmic changes resulting from activation and not to any special influence exerted by the spermatozoön on the metabolism.

The following graph represents the oxygen consumption of *Chætopterus* eggs during the 7 hours following activation and fertilization.

In this graph, the rate of oxygen consumption has been plotted against time, the initial respiration of the unfertilized eggs being considered as 100 per cent. The individual points on the curves represent average values obtained from 14 experiments.

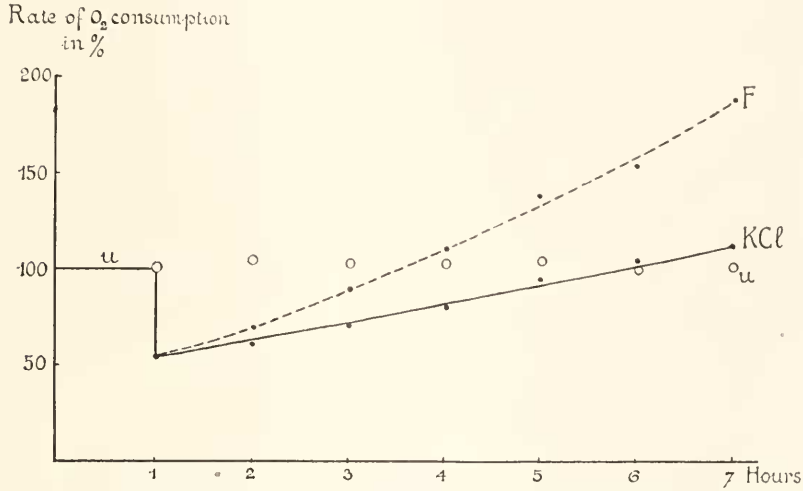

FIG. 1. Oxygen consumption of *Chætopterus* eggs during the seven hours following activation and fertilization. Ordinates: rate of oxygen consumption; abscissæ, hours. The curve *F* represents the oxygen consumption of fertilized eggs; the curve KCl represents that of eggs treated with KCl; and the circles, *u*, represent that for unfertilized eggs.

It is easy to see that, as stated before, the oxygen consumption of the unfertilized eggs (indicated by large circles) remains constant throughout the whole experimental period. It is evident too that respiration increases after the drop in oxygen consumption occurring after fertilization or activation, but obviously this increase is much stronger and faster in the case of the fertilized eggs than it is for the activated eggs: the fertilized eggs reach the initial level after 3½ hours, i.e. at an early blastula stage. The respiration rate increases steadily later on and is almost 80 per cent higher at the end of the experiment than the oxygen uptake of the unfertilized eggs. On the other hand, the activated eggs differentiating without cleavage reach the initial rate only after 6 hours instead of 3½. The fact that the same difference in the respiration of two sets of eggs was found regularly in all the experiments indicates beyond doubt that the observed fact is really significant.

It was possible that the difference might be linked to a depressive effect on the metabolism of the added KCl which was present during the whole experiment. In order to check this possibility, 4 series of

control experiments were run in which the respiration of activated eggs was measured both in presence of KCl in excess as before and after repeated washings (10 times). In other manometers, the oxygen consumption of normal fertilized eggs was compared with the metabolism of similar eggs to which 5 per cent isotonic KCl was added 10 minutes after fertilization. All these control experiments showed that the presence or the absence of KCl in that concentration does not exert any significant effect on the curves. In both cases, the initial respiratory level was reached after 3½ hours in the case of the fertilized eggs and after 6 hours for the activated ones. It seems, however, possible that KCl slightly enhances the absolute O_2 consumption of the eggs. Obviously, the differences observed in the metabolic rate of eggs differentiating without cleavage and of fertilized eggs is somehow linked to their different type of development and not to a depressive effect of KCl on the metabolism.

It was also of some interest to see how the thymonucleic acid synthesis, as a chemical index of the mitotic activity, would compare in fertilized eggs and in eggs differentiating without cleavage. The eggs were therefore taken out of the manometer vessels and preserved in acetone until a sufficient amount of material (about 2 grams) could be collected. The thymonucleic acid content of unfertilized, activated and fertilized eggs was determined by Dische's (1930) colorimetric micromethod. Three estimations could be made. These showed that while the unfertilized eggs contained, as was expected, only traces of thymonucleic acid, the activated eggs had a content in this substance of about 0.35 milligrams per gram wet weight and the fertilized eggs of 1 milligram per gram wet weight. The thymonucleic acid synthesized in 8 hours during differentiation without cleavage amounts thus only to 30 per cent of the amount produced in the fertilized eggs during that period.

Discussion

The curve we obtained for fertilized eggs closely resembles the one published by Whitaker (1933), although its slope is somewhat steeper. In Whitaker's experiments, the unfertilized egg rate is reached by the fertilized eggs in 4½ hours and the increase over that rate at the end of 7 hours of the experiment is only 30 per cent. This difference is very likely due to the higher temperature (24.8° instead of 22°) at which our experiments were carried out: ciliary activity had just begun after 6 hours in Whitaker's case while we noticed swimming blastulae after 4½ hours. It seems therefore probable that conditions similar to those described by Tyler (1936*a*, *b*) in his work on temperature coefficients of developmental processes and cellular respiration prevail

also in *Chætopterus* eggs. It is very probable that the reduced metabolic activity of the eggs differentiating without cleavage is, likewise, somehow linked with their slower development. We have already seen that the control experiments rule out the possibility that the observed difference should result from an inhibition effect of KCl on the eggs' respiration. There is also no doubt that the development of the eggs differentiating without cleavage is slowed down to a considerable extent: for instance, at the end of the experiments the fertilized eggs had turned into actively swimming larvæ while the activated eggs develop cilia only much later. Likewise, the increase of nuclear material, as indicated by the thymonucleic acid estimations, goes on at a much slower rate in the eggs differentiating without cleavage than in the fertilized ones. It is therefore likely that both the reduced oxygen uptake and the slower development are linked together; such a conclusion could support Tyler's opinion that part of the energy available in the egg is needed for the growth and differentiation processes taking place during development. Recent findings by Runnström (1933), J. Brachet (1935), Privolnev (1936), Stefanelli (1937) that the respiration undergoes cyclic changes during mitosis in eggs of different species are also in good agreement with our observations. It must be pointed out, however, that the interpretation of the results is complicated by a special factor; namely, that the activated eggs remain unicellular while the fertilized ones cleave into many cells. It is, of course, by no means impossible that oxidation processes might occur at the surfaces between the different blastomeres. Against such an interpretation, however, two facts can be cited; namely, that there is no direct relationship between the number of the blastomeres and the oxygen uptake of the egg (*cf.* Needham, 1931) and that the respiration of small marine invertebrate eggs is over a wide range independent of the O_2 tension in the surrounding medium.

Summary

1. The oxygen consumption of unfertilized *Chætopterus* eggs drops in the same proportion whether they are fertilized or activated with KCl.

2. The increase in respiratory rate after that initial drop is much faster in fertilized eggs than in activated (differentiating without cleavage) eggs.

3. The lower respiratory activity of the activated eggs is linked to their special type of development.

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